A New Device for Preparing Subunits of Myxovirus

Since Hoyle (1952) reported on ether treatment of Influenza virus for preparing subunits of the virus, the method has been used by several workers to study the properties of the subunits of Influenza virus. (Schäfer and Zillig, 1954; Lief and Henle, 1956; Mizutani, 1958) However, the hamagglutinating titer of the subunits preparation thus obtained was, in our experiences, several times lower than the original titer of the virus suspension employed and the method could not be applied to other myxoviruses, HVJ and NDV, since these viruses are too much sensitive to ether.

During studies on the effect of HVJ on phospholipids of red cells, it was unexpectedly found that when the virus suspension of HVJ was shaken with ether in the presence of a surface-active reagent, Emasol 1130*, the virus particles were effectively disintegrated into their subunits.

HVJ was purified from the infected chorioallantoic fluid by the differential centrifugation. (Fukai and Suzuki, 1955) To 4 ml of the purified HVJ suspension in isotonic saline (12,800 HA/0.5 ml) were added various amounts of Emasol. Then the suspensions were shaken with the same volume of ether for 10 minutes in an ice bath. The aqueous layer was separated by centrifugation, removed and diluted 4 times with isotonic saline to minimize unfavorable effects of ether. An aliquot of the diluted layer was estimated for the hemagglutinating titer and the other was centrifuged at 20,000 rpm for 20 minutes (Spinco L, No. 40 rotor). After the

Table 1 Effect of Emasol 1130 on ether disintegration of HVJ.

	Emasol amount*1					Control (no ether treatment)
	0 mg	5 mg	10 mg	20 mg	30 mg	
Before centrifugation	640 HA*2	5,120	10,240	5,120	1,280	4,000±
Supernatant after 20,000 rpm centrifugation	500	4,000	8,000	4,000	1,000	<500
Sediment after 20,000 rpm centrifugation*3	<500	500±	<500	1,000	<500	4,000±

*1 Emasol was diluted with distilled water to a concentration of 50 mg/ml, and the indicated amounts were added to the virus suspension.

*2 HA titer is expressed as reciprocal of viral dilution at agglutinating titration end point against 0.5 ml of 0.5 per cent fowl red cell suspension.

*3 The sediment was resuspended in the original volume.

* Emasol 1130 is a non-ion surface-active ragent (poly-oxyethylen-sorbitan monolourate), which properties is similar to Tween 20, and was kindly supplied from KAO soap Co., Ltd. Japan.

pellets were resuspended in the original volume of saline, the hemagglutinating titer of the supernatant and the sediment fraction were determined. Four ml of the untreated HVJ suspension was used as control, diluted and centrifuged similarly.

The results are presented in Table 1. When an optimal concentration of Emasol was employed, three times hemagglutinating titer of the control was obtained, while the preparation by the ether treatment in the absence of Emasol showed 1/4 to 1/5 of the control titer. Most of the hemagglutinating agent obtained by Emasol-ether treatment remained in the supernatant after the high speed centrifugation, indicating that the treatment disintegrated the virus particles into a greater number of small subunits. On the contrary, the major portion of the hemagglutinating agent in the control sample was sedimented by high speed centrifugation. The treatment of HVJ with ether for longer period did not produced more the subunits. Emasol had no effect on agglutination of red cells at the concentrations employed.

The treatment was also applied to Influenza virus, PR8 strain, and Newcastle Disease Virus, Osaka strain. Purified Influenza virus (40,000 HA/0.5 ml) and NDV (12,000 HA/0.5 ml) were similarly treated with ether in the presence of Emasol 1130. The results are presented in Table 2 and 3.

Table 2. Effect of Emasol 1130 on ether disintegration of influenza virus, PR8 strain.

	Emasol amount					Control (no ether treatment)
	0 mg	1 mg	2 mg	5 mg	8 mg	
HA. (Before high speed centrifugation)	2,560	10,240	10,240	5,120	2,560	10,240

3 ml of purified virus (40,000 HA/0.5 ml) was used.

Table 3. Effect of Emasol 1130 on ether disintegration of NDV.

	Emasol amount				Control (no ether treatment)
	0 mg	5 mg	10 mg	20 mg	
HA (before high speed centrifugation.)	1,200	19,200	9,600±	4,800	4,800±

3 ml of purified NDV (12,000 HA/0.5 ml) was used.

In these cases also, it was shown that, using the optimal concentration of Emasol, a higher hemagglutinating titer was obtained than that of the control, though in Inflnenza virus sample as much as the control, whereas by ether treatment without Emasol a far lower titer obtained. The test for NDV indicated that most of the hemagglutinating agents in the treated sample were in the supernatant after high speed centrifugaion at 20,000 rpm for 20 minutes. In preparing subunits of Influenza virus, the optimal concentration of Emasol was lower than that for the other two viruses and the titer obtained was relatively lower. In this virus, fractionation by high speed centrifugation showed an almost

equal distribution of hemaggluting titer between the supernatant and the sediment. This seeems to be possibly due to the liberated hemagglutinating agent being still a large size (sedimentation constant of ca. 300 S by ultracenirifugal analysis).

From above experiments, the optimal concentrations of Emasol 1130 for preparing subunits of myxoviruses are as follows: 1 mg for 10,000 HA of HVJ, 1 mg for 100,000 HA of Influenza and 0.5 mg for 10,000 HA of NDV. For preparing subunits by the method, it is not advisable to employ too much concentrated virus per ml.

Thus the present method have given a simple and successful procedure for preparing of subunits of myxoviruses.

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